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The Effect of Energy Supplementation on Intake and Utilisation Efficiency of Urea-treated Low-quality Roughage in Sheep
II. Rumen Kinetics and Acetate Clearance Rate

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ABSTRACT : Inadequate supply of glucose or glucogenic substrates to the body tissues can affect metabolism of absorbed acetogenic metabolites from the gut and therefore, influence feed intake in ruminants. This study investigated the effect of energy supplementation on rumen kinetics in the gut, and the acetate clearance rate in the body tissues of sheep fed low quality basal roughage. A basal diet consisting of urea-treated mixture of wheaten chaff and barley straw (3:1 DM) containing 22.2 g N/kg DM was used. Four Merino cross wethers weighing 45±4.38 kg fitted with permanent rumen and abomasal cannulae were allocated to four treatments in a 4×4 LSD. The treatments were basal diet (E_0), or basal diet supplemented with sucrose (112.5 g/d) administered intraruminally (E_R), abomasally (E_A), or via both routes (50:50) (E_{RA}). There was no difference ($p>0.05$) in the rumen liquid kinetics parameters between the four dietary treatments. However, there was a trend of animals supplemented with sucrose wholly or partly through the abomasum having lower faecal DM and therefore poor pellet formation, and low pH. Although the glucogenic potential of the fermentation products absorbed from the rumen was increased ($p<0.001$) by intra-ruminal supplementation with sucrose (E_R and E_{RA}), there was no significant difference ($p<0.05$) in acetate clearance rate between the four dietary treatments. (**Key Words :** Acetate Clearance Rate, Glucogenic Potential, Rumen Liquid Kinetics, Substrate Cycle)

INTRODUCTION

Low quality roughages such as cereal straw and stover are high in fibre but low in readily fermentable carbohydrates, nitrogen (N) and minerals such as sulphur (Leng, 1990). As a result they are not only poorly digested in the rumen but their fermentation pattern is characterized by a high proportion of acetogenic (acetate and butyrate) relative to glucogenic substrates. As a result their capacity to supply the body tissues with glucose either directly through intestinal absorption or indirectly via propionate fermentation in the rumen is generally low. This is in spite of glucose or glucogenic substrates such as propionate or amino acids being required for tissue metabolism of acetogenic substrates which are the major digestion products derived from the fermentation of high-fibre, low-protein basal roughage that form the highest proportion of ruminant feed, especially in the tropics (Preston and Leng, 1987; Leng, 1990; Illius and Jessop, 1996). As a result low quality basal roughage is poorly utilized by ruminants mainly due to a combination of factors such as high cell wall constituents (CWC), low microbial growth and fermentation activity in the rumen, and inadequate supply of glucose or glucogenic substrates, especially propionate and amino acids at the tissue metabolism level (Leng, 1990; Illius and Jessop, 1996; Ferrell et al., 1999).

Energy supplementation has been reported as being variably beneficial in enhancing digestibility and intake of basal roughage dry matter (DM) and organic matter (OM), with some of the benefits being mediated at the rumen and/or tissue metabolism level (Ørskov, 1986; Lee et al., 1987; Fonseca et al., 2001; Royes et al., 2001). While readily digestible carbohydrate supplements can impact negatively on the digestibility of the basal roughage, they may also increase the fermentable substrate carbon in the rumen thus supplying rumen microbes with energy. This is likely to enhance microbial protein supply to the body tissues, especially when the rumen ammonia is not limiting. In addition, the dietary energy supplement increases

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propionate absorption in the rumen which is likely to stimulate higher glucose synthesis in the body tissues, and therefore enhancing metabolism of acetogenic substrates, mainly acetate at the tissue level, and possibly stimulating higher basal roughage intake. It is hypothesized that on quantitative basis, intestinal digestion of readily fermentable carbohydrate yields more absorbed glucose compared to the same quantity of carbohydrate when rumenally fermented. It is therefore anticipated that the later route of carbohydrate supplementation would delivery more glucose to the body tissues and as a result lead to a higher acetate clearance rate in the body tissues. This should translate to higher intake of low quality roughage whose digestion in the rumen is mainly characterized by acetate type of fermentation. Acetate clearance rate in the body tissues has been suggested as a possible index of the body's glucogenic potential, and therefore can be used as an indirect measure of the body's efficiency to metabolise acetogenic substrates (Cronje et al., 1991).

The objective of the present study was to investigate the effect *in vivo* of intraruminal and intra-abomasal supplementation with sucrose in increasing the absorption of glucogenic products (fermentation and digestion) from the gut. This is with a view of increasing the metabolic efficiency of acetogenic substrates in the body tissues as measured by acetate clearance test.

MATERIALS AND METHODS

Animals, their management and experimental design

Four (4) Border Leicester×Merino crossbred wethers weighing 45.0±4.38 kg, each fitted with a permanent rumen cannula and an abomasal cannula were re-located to the animal house and housed in individual pens. A feeding trial involving the four wethers and four treatments was carried out in four periods in a balanced 4×4 Latin square design as described in Part I.

Diets and the application of treatments to animals

The diets and the application of treatments are as described in paper I. In brief, the basal diet consisted of wheaten chaff (91% DM, 0.71% N)/barley straw (93% DM, 0.55% N) (3:1 DM basis) treated with urea at 2.5% DM (2.5 kg urea in 20 L warm water), and put in synthetic gunny bags, compacted and stored at room temperature until the time of feeding. The animals were offered the basal diet *ad lib.* and supplemented with equal amounts of metabolisable energy (ME) in the form of sucrose. The fresh energy supplement was prepared daily by dissolving sucrose (375 g) in 750 ml of warm tap water to make 1 L of sugar solution. About 300 ml of this solution was administered to each animal (except control) intraruminally (E_R), abomasally (E_A) or via both routes (50:50) (E_{RA}) in two doses of 150 ml at 09.00 and 16.00 h each day with each animal receiving approximately 112.5 g sucrose per day.

Rumen liquid kinetics

Rumen fluid volume, outflow rate and the time taken for half of the rumen fluid volume to be removed and replaced ($T_{1/2}$) were determined using Cr-EDTA complex as the marker for the liquid phase (Downes and McDonald, 1964). Samples of rumen fluid (25 ml) were collected before the marker injection and thereafter at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 21 and 24 h. About 15 ml of the fresh rumen fluid was used to determine the rumen kinetics. This portion (15 ml) was acidified with 0.4 ml of 18 M H_2SO_4 and stored at -20°C for Cr, ammonia-N and VFA analysis. The Cr concentration in the supernatant of the centrifuged (3,000× g) rumen fluid samples was determined after the samples were digested with perchloric acid ($HCLO_4$)/H_2O_2 (7:3 v/v). The Cr concentration was analysed using an Inductively Coupled Plasma Optical Spectrometer (ICP-OES) (wavelength 175-785 nm). The dilution of Cr over time in the rumen was assumed to obey 1st order kinetics ($C_t = C_0e^{-kt}$) where:-C_t is the concentration of Cr at time (t), C_0 is the concentration of Cr (mg/L) at zero time, and *k* is the rate constant (slope of the regression line).

Acetate clearance rate

Acetate clearance rate was determined using the method first proposed by Weston (1966) and later modified by Cronje (1987). The animals were catheterized in the jugular vein a day before in readiness for injection of pre-warmed (37-40°C) sodium acetate adjusted to pH 7.40 with 1 M NaOH. A single dose (4 mmol/kg body weight) in 50 ml of de-ionised water was injected into the jugular vein over a period of 2-3 min. A 5 ml blood sample was drawn from the jugular vein into a heparinised tube (to avoid clotting) before acetate injection, and 30, 60, 90, 120 and 150 minutes post injection to monitor the changes in the level of acetate in the blood with time. The blood sample drawn before acetate injection was used to establish the background (basal) concentration of acetate in the animal prior to intravenous acetate loading. During the 2.5-h collection period, the blood samples were kept in a cooled ice-box and thereafter centrifuged at 3,000×g for 15 min to separate the blood cells from the plasma. The cellular portion was discarded while the plasma supernatant (3 ml) was decanted and then deproteinised by adding 0.3 ml of 50% (w/v) sulphosalicyclic acid, mixed thoroughly and centrifuged. The clear deproteinised plasma was decanted and stored at -20°C for later analysis of acetate.

The plasma acetate concentration was determined using gas liquid chromatography (Model CP-3800GC) with iso-caproic acid as the internal standard (Erwin et al., 1961).

Table 1. The liquid kinetics in the rumen of sheep fed urea-treated low quality basal roughage and supplemented with sucrose through the rumen or abomasum

Parameter	Dietary treatments				
	E_0	E_R	E_A	E_{RA}	SEM
Rumen fluid volume (L)	11.3	13.0	8.4	9.6	1.84 (ns)
Rate constant ($\times 10^{-2}$)(/h)	5.51	6.99	4.15	5.08	0.00997 (ns)
Outflow rate (L/d)	11.3	14.7	8.9	11.4	2.37 (ns)
$T_{1/2}$ (h)	13.1	9.9	14.2	11.5	3.08 (ns)

E_0, no sucrose supplementation (control), or sucrose supplement administered through the rumen (E_R), abomasum (E_A) or both routes in equal amounts (E_{RA}).

The acetate (Ac) concentration (mmol/L) in the plasma of blood samples collected post injection (Ac_t) was corrected for the pre-injection concentration of acetate (Ac_o), transformed to a natural logarithm scale and then regressed against time, i.e. ln (Ac_t-Ac_o) vs. t. Where Ac_t is the concentration of acetate at time *t* post injection, Ac_o is the plasma acetate concentration pre-injection and *t* is the sampling time. The volume of distribution of the acetate load (L), acetate clearance rate constant (*k*) (/min) and the time required for the injected acetate dose in the blood of the animal to be reduced by half ($T_{1/2}$) were determined.

Faecal characteristics

For the determination of faecal pH, freshly voided faeces was weighed, mixed with water (1:2 fresh weight basis) and then pulverized with a glass rod to make a slurry. The pH was determined with a glass electrode pH meter. The DM content of faeces was estimated on the dried samples (AOAC, 1990). The faeces were also assessed subjectively for consistency and faecal pellet formation and classified as normal (having well formed pellets), moist and lumpy (i.e. devoid any well formed pellets), or intermediate.

Statistical analysis

The data were analyzed by ANOVA for a 4×4 Latin square design using Minitab computer statistical software (Ryan et al., 1985), and separation of means done using the Tukey test at 5%.

RESULTS

Liquid kinetics in the rumen

Animals supplemented with sucrose wholly through the rumen had the largest rumen fluid volume, highest dilution rate (0.007/h) and outflow rate (14.7 L/d), and the shortest rumen $T_{1/2}$ (9.9 h). However, the differences between treatments were not significant ($p>0.05$) (Table 1).

Faecal characteristics

There was a significant difference ($p<0.001$) between the faecal DM content of animals on the four dietary treatments. The faecal DM content of the animals receiving the sucrose supplement through the abomasum was lower ($p<0.05$) than that of animals on control diet or receiving sucrose supplement intraruminal. However, the fecal DM of animals on abomasal sucrose supplementation was not different ($p>0.05$) from that of animals supplemented through both intra-ruminal and abomasal routes (E_{RA}). There was also a trend towards low faecal pH in animals supplemented with sucrose wholly or partially through the abomasum compared to that of animals on the control or supplemented wholly intraruminally (Table 2). The faeces of the animals supplemented with sucrose wholly through the abomasum, and to some extent those receiving the supplement through both rumen and abomasum, showed poor pellet formation (Figure 1).

Acetate clearance

There was no significant difference ($p>0.05$) in clearance rate constant (*k*), acetate clearance rate (mol/h), and clearance half-life ($T_{1/2}$) between the four treatments (Table 3). There was however, a trend whereby animals supplemented with sucrose entirely through the abomasum generally had the highest mean clearance rate constant (13×10^{-3}/min) and acetate clearance rate (16.0×10^{-2} mol/h) and also the shortest clearance half-life (0.94 h), even though

Table 2. The faecal characteristics of sheep fed urea-treated low quality basal roughage (E_0) and supplemented with sucrose through the rumen (E_R), abomasum (E_A) or both routes 50:50 (E_{RA})

Parameter	Dietary treatments				
	E_0	E_R	E_A	E_{RA}	SEM
Faecal DM (g/kgDM)	339^b	322^b	273^a	290^{ab}	12.7 (***)
Faecal pH*	8.69	8.70	4.91	6.96	NA
Visual appraisal	Normal	Normal	Very moist	Intermediate	-

* Means for period 1 only and hence data not analysed statistically (NA); means within row with different superscripts differ ($p<0.05$).

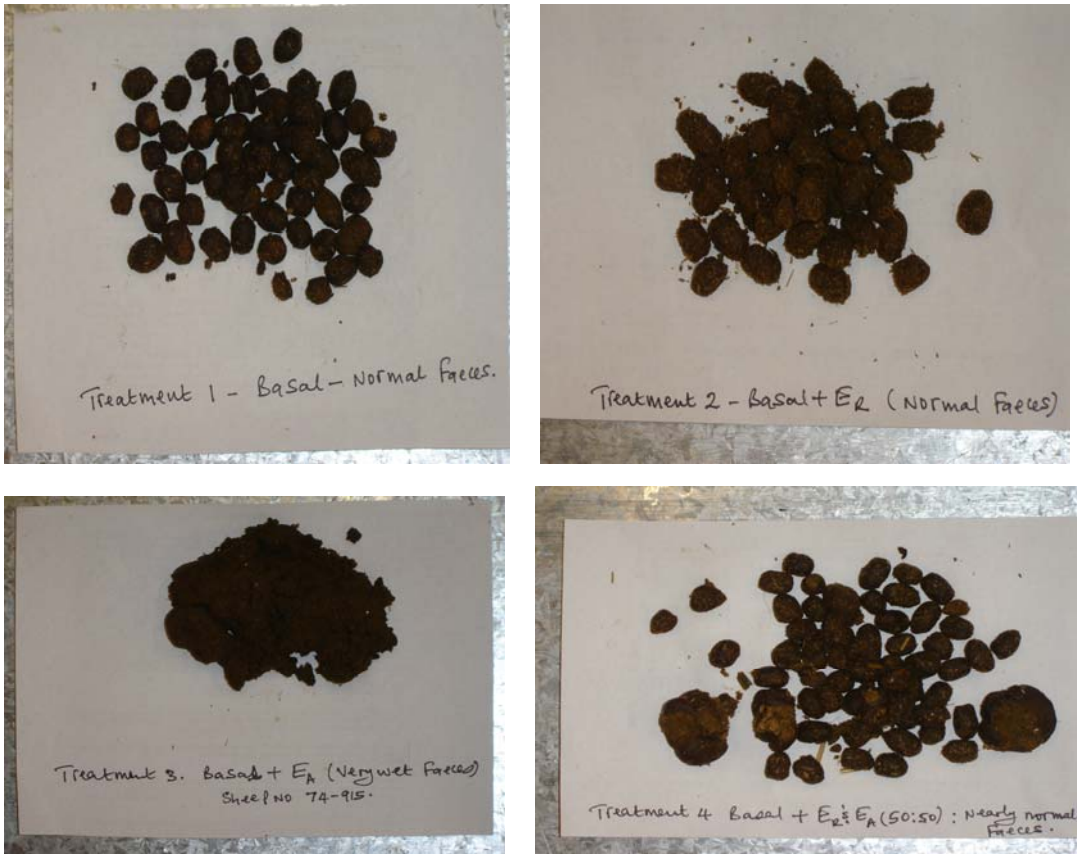

Figure 1. The faeces of sheep fed urea-treated basal roughage (E_0) and supplemented with sucrose intraruminally (E_R), abomasally (E_A) or both routes 50:50 (E_{RA}).

these were not significantly different from those of the other three dietary treatments (i.e. E_0, E_R and E_{RA}).

DISCUSSION

Faecal characteristics

The trend in faecal pH indicated that there was extensive hindgut fermentation activity in animals supplemented with sucrose entirely through the abomasum (E_A), and also, to a lesser extent those supplemented via the intraruminal and abomasal routes (E_{RA}). Animals supplemented with sucrose through the abomasum also produced very moist faeces with low DM content, poor consistency and that was almost devoid of any faecal pellet formation, besides being moderately acidic (pH 4.91). All these changes in faecal characteristics point to a possible impairment in fluid exchange in the hindgut between the digesta and the body tissues, possibly occasioned by changes in osmotic potential. These changes were attributed to the presence of soluble sugars in the hindgut that had passed through the small intestines without being effectively digested and/or absorbed. The fermentation of these sugars in the hindgut produced VFA that was responsible for the acidity resulting in the low faecal pH. Furthermore, some of the unfermented sugars and VFA could have caused osmotic changes in the gut that lead to an influx of water into the lumen of the hindgut resulting in moist faeces with very poor faecal pellet formation. Ørskov et al. (1972) attributed such hindgut fermentation of sucrose to low sucrase (invertase) activity in the small intestines of

Table 3. Acetate clearance in the body of sheep fed urea-treated low quality basal roughage and supplemented with sucrose energy

Parameter	Dietary treatments				
	E_0	E_R	E_A	E_{RA}	SEM
Clearance rate constant ($\times 10^{-3}$)(/min)	9.00	8.67	13.0	6.50	0.00280 (ns)
Acetate clearance rate ($\times 10^{-2}$)(mol/h)	9.78	8.97	16.0	6.95	0.0306 (ns)
Clearance half-life ($T_{1/2}$)(h)	1.30	2.61	0.94	1.93	0.55 (ns)

E_0, no sucrose supplementation (control), or sucrose supplement administered through the rumen (E_R), abomasum (E_A) or both routes in equal amounts (E_{RA}).

ruminants, an occurrence that has also been reported by others (Walker, 1959; Siddons, 1968).

During the entire trial that lasted for nearly five months it was observed that even in the abomasally supplemented animals that produced faeces with the lowest DM and very poor pellet formation, the condition of the faeces tended to improve over time. This suggests that over time the animals were somehow able to adapt to the presence of large quantity of sucrose in the gut (mainly small intestines) by either increasing sucrase activity and/or increasing absorptive capacity of sucrose digestion products (glucose and fructose). Such adaptation of the gut to large inflow of starch (or glucose) has been speculated on the basis of *in vitro* studies (Zhao et al., 1998). Moreover, a positive adjustment in glucose absorption from the gut may also be in response to increased demand of this substrate at the tissue level. For example, there are indications from *in vitro* studies with isolated enterocytes of dairy cattle that the rate of glucose absorption from the gut may be increased to match glucose requirements at the tissue metabolism level, especially during early lactation (Okine et al., 1994, 1995). Such adaptation may be partly realised through the normal passive transfer of glucose being complemented by the active sodium-dependent glucose transportation (SGLT1) system that requires the participation of energy in form of ATP (Zhao et al., 1999).

Liquid kinetics in the rumen

The lack of significant difference between treatments in rumen kinetic parameters was probably due to the large variations in the estimates of rumen fluid turnover among the animals. However, the results showed a general trend of the animals supplemented with sucrose entirely through the rumen (E_R) generally having the highest estimate of rumen fluid volume, rate constant, and outflow rate but generally low $T_{1/2}$. These observations are consistent with a possible increase of fluid in the rumen originating from higher water intake or influx of the fluid from body tissues. This could have occurred if the presence of supplemental sucrose in the rumen of these animals raised the osmotic potential of the rumen fluid.

Animals that received sucrose supplement entirely through the abomasum produced very moist faeces, a situation that could have lead them to increase their water intake so as to compensate for the excessive faecal water loss and thus avoid tissue dehydration. Any such increase in water intake would be expected to result in significant changes in the rumen kinetics, including increase in dilution of rumen contents. It was suggested that this may have been partly responsible for the rather low total VFA concentration in the rumen of these animals in spite of having an apparently high dietary intake (Part I). However, it is highly unlikely that the extra fluid in the rumen was removed by outflow *per se* as this would have increased rumen kinetic parameters especially, dilution rate constant and even outflow rate in these animals, which apparently is not supported by the rumen kinetics results. This suggests that such water may have been absorbed across the rumen wall and subsequently voided in the urine, rather than leaving the rumen via the reticulo-omasal orifice to the lower part of the gut. Though the water intake of the experimental animals was not monitored during the trial, this hypothesis is partly supported by the observation that animals supplemented with sucrose entirely through the abomasum (E_A) generally had the highest mean daily urinary output. The mean daily urine output in the animals on the four dietary treatments during the four periods was as follows: 780 (E_0), 790 (E_R), 890 (E_A) and 830 ml/d (E_{RA}).

Acetate clearance rate

It seems that the significantly higher glucogenic potential that was predicated in animals on dietary treatments E_R and E_{RA} on the basis of rumen fermentation parameters did not necessarily translate to a more rapid acetate clearance in the body tissues. It is probable that sucrose supplementation intraruminally (or abomasally) may have enhanced glucogenic potential in the body tissues and increased their capacity to metabolise acetate, but the differences masked by the large variation in the estimate of acetate clearance parameters, especially the clearance rate constant obtained in this study. Large variation in acetate clearance rate is not unique to this study as it has been reported before (Fonseca et al., 2001).

Propionate is considered to be highly glucogenic and when its proportion in total VFA is high, it could be expected to increase the gluconeogenesis in the liver and therefore enhancing glucose level in the body (Wolin, 1981; Ørskov, 1982; Preston and Leng, 1987). On the other hand acetogenic substrates, especially acetate, are mainly used in the tissues to meet the immediate energy (ATP) requirements of the animal with any surplus being conserved as fat (Cronje et al., 1991). However, an efficient utilization of acetate for oxidative metabolism to generate ATP is dependent on adequate amounts of oxaloacetate to prime the TCA cycle (Brockman, 1993). Similarly, the synthesis of long chain fatty acids (LCFA) in the adipose tissue so as to conserve the surplus energy as fat is dependent on the supply of reduced nicotinamide adenine dinucleotide phosphate (NADPH) which is mainly obtained from oxidation of glucose via the pentose phosphate pathway (Crabtree et al., 1987; Cronje et al., 1991). Glucose is also required to form glycerol that is used in the formation of triglycerides in the adipose tissue.

Any acetate not utilized for ATP production or conserved as fat (due to shortage of glucose) may be wastefully oxidized to heat through the "substrate cycle"

leading to the high heat increment that has for a long time normally been associated with intake of roughage in ruminants (Blaxter, 1967; MacRae and Lobley, 1982; MacRae et al., 1985; Crabtree et al., 1987, 1990). Inadequate glucose supply therefore reduces the capacity of body tissues to metabolise the absorbed acetate leading to its accumulation in the tissues and in the process constraining voluntary intake of roughage in ruminants, especially those in hot tropical environments (Preston and Leng, 1987; Cronje et al., 1991). However, the intake of roughage by animals in cool temperate areas is not adversely affected as the heat generated from the "substrate cycle" can be used to keep them warm. It follows therefore that an enhanced glucose (or glucogenic substrates) supply in the body can expected to lead to a higher efficiency in the utilization of acetogenic substrates (Brockman, 1993), and in the process stimulating higher voluntary intake (Wolin, 1981), especially for animals in the tropics. The increase in voluntary intake that is normally associated with higher protein/energy ratio is attributed to the enhanced glucose supply from the glucogenic amino acids in the protein (Egan, 1977).

Sucrose supplementation wholly or partly through the rumen (E_R and E_{RA}) generally resulted in a higher predicted glucogenic index (G/E) than the control or abomasally supplemented animals (Migwi et al., see Part I). It is also quite possible that animals supplemented with sucrose abomasally though having a fermentation pattern characterized by high acetate and therefore much lower propionate/acetate and glucogenic potential index (G/E ratio) than the control (Part I), may have also benefited from intestinal digestion of sucrose and/or propionate absorption from hindgut and therefore a higher supply of glucose to the body tissues. However, this proposition was not supported by the results of acetate clearance tests as there was no significant difference in acetate clearance rate between the animals on the four dietary treatments. This was rather surprising, especially when the rumen fermentation results indicated a higher glucogenic potential index for intraruminally supplemented animals (Migwi et al., see Part I). Similarly, the abomasally supplemented animals (E_A) were expected to have high tissue glucose from the intestinal absorption. This however, presumes that all the sucrose was hydrolysed by enzymes in the small intestines and absorbed into the body tissues as glucose and fructose, though it has been reported that sucrase (invertase) enzyme activity in the small intestines of ruminants is rather low (Siddons, 1968; Ørskov et al., 1972). It is also noteworthy that this trial was conducted in March to July which coincided with autumn-winter period in the high altitude Tablelands of New England in New South Wales where ambient temperatures are generally low during that season. It has been reported that due to climatic difference the response to (protein) supplementation by ruminants in the temperate areas is generally lower than in the tropics (Preston and Leng, 1987; Leng, 1990).

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